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In re Application of	
Application Number: 08-149,508	Filed: 11-9-93
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US005750376A

United States Patent [19]

Weiss et al.

[11] Patent Number: 5,750,376

[45] Date of Patent: May 12, 1998

[54] **IN VITRO GROWTH AND PROLIFERATION OF GENETICALLY MODIFIED MULTIPOTENT NEURAL STEM CELLS AND THEIR PROGENY**

[75] Inventors: **Samuel Weiss**; **Brent Reynolds**, both of Alberta, Canada; **Joseph P. Hammang**; **E. Edward Baetge**, both of Barrington, R.I.

[73] Assignee: **NeuroSpheres Holdings Ltd.**, Calgary, Canada

[21] Appl. No.: **483,122**

[22] Filed: **Jun. 7, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 270,412, Jul. 5, 1994, abandoned, Ser. No. 385,404, Feb. 7, 1995, abandoned, Ser. No. 359,945, Dec. 20, 1994, abandoned, Ser. No. 376,062, Jan. 20, 1995, abandoned, Ser. No. 149,508, Nov. 9, 1993, abandoned, Ser. No. 311,099, Sep. 23, 1994, abandoned, and Ser. No. 338,730, Nov. 14, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, said Ser. No. 385,404, Feb. 7, 1995, abandoned, is a continuation of Ser. No. 961,813, Oct. 16, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, said Ser. No. 359,345, Dec. 20, 1994, abandoned, is a continuation of Ser. No. 221,655, Apr. 1, 1994, abandoned, which is a continuation of Ser. No. 967,622, Oct. 28, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, said Ser. No. 376,062, Jan. 20, 1995, abandoned, is a continuation of Ser. No. 10,829, Jan. 29, 1993, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, said Ser. No. 270,412, Jul. 5, 1994, abandoned, Ser. No. 149,508, Nov. 9, 1993, abandoned, and Ser. No. 311,099, Sep. 23, 1994, abandoned, each is a continuation-in-part of Ser. No.726,812, Jul. 8, 1991, abandoned.

[51] **Int. Cl.**[6] **C12N 5/00**; C12N 5/08; C12N 5/10; C12P 1/00

[52] **U.S. Cl.** **435/69.52**; 435/69.1; 435/172.3; 435/325; 435/368; 435/377; 435/384; 435/392; 435/395

[58] **Field of Search** 435/240.2, 172.3, 435/69.1, 69.52, 325, 368, 377, 384, 392, 395

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[57] **ABSTRACT**

A method for producing genetically modified neural cells comprises culturing cells derived from embryonic, juvenile, or adult mammalian neural tissue with one or more growth factors that induce multipotent neural stem cells to proliferate and produce multipotent neural stem cell progeny which include more daughter multipotent neural stem cells and undifferentiated progeny that are capable of differentiating into neurons, astrocytes, and oligodendrocytes. The proliferating neural cells can be transfected with exogenous DNA to produce genetically modified neural stem cell progeny. The genetic modification can be for the production of biologically useful proteins such as growth factor products, growth factor receptors, neurotransmitters, neurotransmitter receptors, neuropeptides and neurotransmitter synthesizing genes. The multipotent neural stem cell progeny can be continuously passaged and proliferation reinitiated in the presence of growth factors to result in an unlimited supply of neural cells for transplantation and other purposes. Culture conditions can be provided that induce the genetically modified multipotent neural stem cell progeny to differentiate into neurons, astrocytes, and oligodendrocytes in vitro.

40 Claims, 3 Drawing Sheets